The enzyme thymidylate synthase (TS, E.C.2.1.1.45) from *Mycobacterium tuberculosis* as a target for novel antitubercular drug development.

Caroline Brancher\textsuperscript{1,2}; Ardala Breda\textsuperscript{1}; Luiz Augusto Basso\textsuperscript{1}; Diógenes Santiago Santos\textsuperscript{1,2}

1. Centro de Pesquisas em Biologia Molecular e Funcional. Instituto Nacional de Ciência e Tecnologia em Tuberculose. Avenida Ipiranga, 6681 Tecnopuc Prédio 92A. Telefone/Fax (51) 3320.3629.

**Introduction**

Tuberculosis (TB) remains a major global health concern. Its causative agent, *Mycobacterium tuberculosis*, has been estimated to infect approximately one-third of the world's population, and approximately 30 million people have died from the disease in the past decade. The World Health Organization estimated a total of 9.27 million new cases of TB and approximately 1.78 million deaths from this disease in 2007, second only to AIDS among infectious diseases. The emergence of drug resistant isolates of *M. tuberculosis*, particularly of multi drug-resistant TB (MDR-TB), defined as resistant to at least isoniazid and rifampicin, imposes a great challenge to public health. Treatment of drug resistant TB requires the administration of second-line drugs that are more toxic and less effective. Thymidylate synthase (TS, EC 2.1.1.45) is a key enzyme for the *de novo* synthesis of DNA and as such a target for development of new medicines against TB. TS is a critical enzyme for DNA replication since it catalyses the *de novo* synthesis of thymidine monophosphate (TMP), a key nucleotide precursor for DNA synthesis.
Methodology

In this work, our specific goals were to amplify, clone and subsequently express the gene \textit{thyA} that encodes \textit{M. tuberculosis} TS protein. Synthetic oligonucleotides were designed and \textit{M. tuberculosis} genomic DNA was used as a template for DNA fragment amplification by PCR. PCR samples were analyzed by agarose gel electrophoresis, and an amplification product compatible with the expected size (792 bp) for the \textit{thyA} sequence was observed. This product was gel-purified and cloned into the pCR-Blunt vector. The next step was subcloning \textit{thyA} into a prokaryotic expression vector. In order to achieve this the DNA fragment was cut by the \textit{NdeI} and \textit{HindIII} restriction enzymes and subcloned in pET-23a(+) expression vector. Expression of the TS protein was than attained with the construct \textit{thyA::pET-23a(+) with different strains of Escherichia coli}, in order to obtain the protein in the soluble fraction for further purification.

Results and Conclusion

We were able to amplify the \textit{thyA} gene of \textit{Mycobacterium tuberculosis} from its total genomic DNA, with the expected size of 792 bp. The amplified gene was correctly cloned into cloning and expression vectors, pCR-Blunt and pET-23a(+). Best protein expression on the soluble fraction was observed with C41(DE3) \textit{E. coli} strain, TB medium, at 30ºC and 0.1mM IPTG induction.

These results will allow protein purification by high-performance liquid chromatography and biochemical assay for TS activity. Future work will also involve enzyme kinetics and thermodynamic studies for detailed characterization of the protein’s biochemical proprieties. These will be important for characterizing the pyrimidine metabolism pathway in mycobacteria and for development of new drugs against TB.

References


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